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TECHNICAL REPORT

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PRINCIPAL INVESTIGATOR: Robert P. Gunsalus, Ph.D.

INSTITUTION: University of California, Los Angeles

GRANT TITLE: Genetic Analysis of Hyperthermophilic Archaebacterial Phenomena

AWARD PERIOD: 1 March, 1991 - 28 February, 1994

OBJECTIVE: To establish and exploit genetic techniques for the study of the extremely hyperthermophilic archaebacterium, Sulfolobus acidocaldarius.

APPROACH: Develop, refine and use genetic methods to analyze basic properties of a model hyperthermophilic archaebacterium, Sulfolobus acidocaldarius.

ACCOMPLISHMENTS:

The results of this project have resulted in several major advances in the development and analysis of genetics systems in the extreme thermophilic archaebacterium, Sulfolobus acidocaldarius. The goals have been to study gene regulation in this group of microbes as essentially nothing has been done previously due to the lack of tools in these organisms. Methods were developed to routinely culture and plate S. acidocaldarius on solid medium (petri plates). Culture conditions were optimized with respect to medium composition, cell environmental conditions, handling and storage of mutant strains, and development of stable temperature controlled incubators. This organism grows optimally at 75-80 degrees C at a pH of 5-6. Commercially available drying ovens were modified to grow cells on plates at temperatures from 75-85 degrees. Cells can not routinely be plated at high efficiency (ca 90%) on any of a number of standardized plating media types. These include a minimal medium containing a mineral salts mixture plus any of several single carbon supplies as well as rich media.

Techniques for performing routine mutant selection and screen were also devised. This has allowed us for the first time to generate and characterize large numbers of auxotrophic strains. We used these techniques to fully establish the biochemical pathway for the biosynthesis of the cellular nucleic acid intermediate, UMP, in S. acidocaldarius.

High temperature methods were also refined to perform enzyme assays for characterization of auxotrophic strains. Enzyme assays for each of the biochemical steps of UMP synthesis from glutamine, CO_2 and ATP were developed for S. acidocaldarius extracts. Analysis of wildtype and Pyr pathway mutants were routinely performed so that we could establish what steps of the UMP biosynthetic pathway are regulated by availability of precursors and/or products. This study establishes that the control of UMP is highly regulated in this thermophilic bacterium and establishes the foundation of future studies to examine the molecular basis of this control.

Methods were developed to mass culture cell material at sufficient levels so that enzyme

purification could be undertaken. One enzyme of the UMP pathway, aspartate carbomyl transferase (ATC) was purified for subsequent biochemical analysis. The longer term goal of this study will be to do reverse genetics in order to obtain the gene for the ATCase enzyme. We wish to characterize its structure relative to the analogous genes in eubacteria and eukaryotes. We also wish to study its regulation. Towards this goal, we N-terminal amino acid sequenced the purified enzyme so that gene probes could be devised and made in order that the gene be cloned and sequenced.

In other studies, the spontaneous mutation rates in Sulfolobus acidocaldarius was examined. A mutator strain was identified and characterized. This exploratory study should allow the future study of gene stability in high temperature bacteria.

SIGNIFICANCE:

Although the study of the hyperthermophilic archaebacteria is still in its infancy, we have proceeded over the past three years to develop classical genetic techniques to address mechanisms of gene control in a model hyperthermophilic archaebacterium, Sulfolobus acidocaldarius. Prior studies with these organisms were limited to those that could be done in liquid culture. This was a serious limitation which had precluded application of cell plating and bacterial genetics approaches such as mutagenesis, isolation of mutant strains, or recovery of genetic recombinants that will enhance a variety of future studies. Projects that are now addressable include the analysis of mutational events, manipulation of individual biosynthetic pathways, and development of molecular-genetic tools for the detailed study of dynamic molecular phenomena of hyperthermophilic archaebacteria.